

# DETERMINATION OF THE OPTIMAL EXPOSURE CONDITIONS THAT PRODUCE MAXIMUM ISOEUGENOL CONCENTRATIONS IN RAINBOW TROUT SKIN-ON FILLET TISSUE

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## Abstract

Rainbow trout were exposed to AQUI-S™ in water temperatures of 7, 12, and 17 °C in one of two static exposure regimens: 1) 14 mg/L AQUI-S™ for 60 min or 2) 34 mg/L AQUI-S™ for 10 min (exposure conditions based on expected use in the U.S.).

At common water temperatures, the tissue concentration of isoeugenol (active ingredient in AQUI-S™) from fish exposed to 14 mg/L AQUI-S™ for 60 min was significantly greater than the isoeugenol concentration in fish exposed to 34 mg/L AQUI-S™ for 10 min ( $P < 0.01$ ). AQUI-S™ exposure regimens and exposure temperatures can significantly impact drug residue concentrations in fillet tissue.

## Introduction

Currently, Finquel® (tricaine methanesulphonate or MS-222) is the only fish anesthetic approved by the U.S. Food and Drug Administration (FDA). Use of this anesthetic is constrained in the U.S. by a 21-day withdrawal period, i.e. fish can not be made available for consumption for 21 days after exposure to the anesthetic. This constraint fosters the need in U.S. public aquaculture for an anesthetic/sedative with a reduced withdrawal time. AQUI-S™ New Zealand LTD is pursuing approval for AQUI-S™ in the U.S.

Attributes of a drug that must be characterized before the drug is approved include 1) characterizing the depletion of a drug's total residues after exposure and 2) characterizing the depletion of a marker residue (the parent compound, a metabolite, or combination of residues that persist for the longest time in the target tissue, e.g. edible fillet tissue). Characterizing marker residue depletion allows the FDA to establish withdrawal times ensuring total residue concentrations reach safe levels.

The objective of this study was to determine the exposure parameters (water temperature, exposure duration, and AQUI-S™ concentration) that would generate the greatest total isoeugenol (active ingredient in AQUI-S™) residues in fish fillet tissue allowing for selection of a marker residue. The water temperatures were chosen to represent the range of temperatures for culturing rainbow trout. The two exposure durations and AQUI-S™ concentrations were chosen to represent the label claims that will be pursued in the U.S., i.e., a "rested harvest" claim and a "general anesthesia" claim.

## Methods



Exposure baths were mixed well before exposure. Water temperature and D.O. were measured before and after exposure.



Three fish were placed in each tank. There were 4 tanks per exposure regime.



Skin-on fillets were taken from each fish immediately after exposure.



Water samples were processed through solid phase extraction columns.

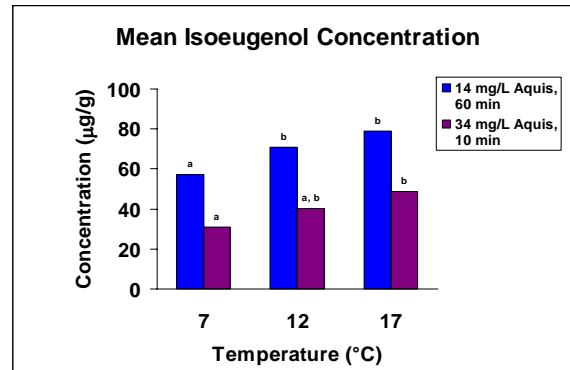


Fillet tissue was homogenized and extracted with acetonitrile. Extracts were processed through solid phase extraction columns.



The isoeugenol concentrations in the tissue and water extracts were determined by HPLC.

## Results



Isoeugenol concentrations in fillets from fish dosed with the low dose were significantly greater than the concentrations in fillets from fish dosed with the high dose ( $P < 0.01$ ). Bars of the same color with a common letter are not statistically different ( $P > 0.05$ ).



## Acknowledgments

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## Conclusion

Maximum isoeugenol residues in fillet tissue from rainbow trout are generated with a "rested harvest" AQUI-S™ exposure regimen in optimum or maximum water temperatures for culturing rainbow trout.